RESPIRATORY LEVEL OF SEA URCHIN EGGS BEFORE AND AFTER FERTILIZATION

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Following Warburg's experiments (1908), it was believed that the respiration of sea urchin eggs increased sharply at the time of fertilization. This phenomenon attracted much attention in the past and a number of papers appeared on the mechanism of respiratory increase at fertilization (*cf.* Runnström, 1949, and Rothschild, 1956, for references). Using the Cartesian diver technique, however, Borei (1948a, 1949) found that oxygen uptake of unfertilized eggs of *Psammechinus miliaris* decreased gradually following removal from the ovary. He assumed that there would be no increase in oxygen uptake if the eggs were fertilized immediately after shedding under natural conditions. According to his finding, the increase in oxygen uptake at fertilization seemed to be a special phenomenon and general interest in it diminished considerably. However, there were some data which threw doubt on the generalization of Borei's result. In the early study on *Arbacia* eggs, Tyler, Ricci and Horowitz (1938) showed that oxygen uptake of unfertilized eggs could remain fairly steady for several days starting shortly after removal of the eggs from the female. Cleland (1950) reported that in oyster eggs there was no gradual decline in oxygen uptake following removal from the ovary. On the other hand Tyler and Humason (1937) found that different batches of unfertilized eggs of the gephyrean worm, *Urechis caupo*, could have different absolute rates of respiration, depending upon their past history (storage time in the "nephridial sacs," etc.), some being higher than, some lower than, and some about the same as, that of the fertilized eggs. The latter showed about the same absolute rate regardless of batch. Because of the importance of Borei's finding, it was considered to be meaningful to repeat his experiments with different species of sea urchins. The present experiments showed somewhat different results from those of Borei. No decline in oxygen uptake of unfertilized eggs could be observed for some time after shedding. Further, the respiratory level of fertilized eggs was much higher than that of unfertilized eggs.

Materials and Methods

Three species of Japanese sea urchins, *Hemicentrotus pulcherrimus*, *Pseudocentrotus depressus* and *Anthocidaris crassispina*, were collected at the Misaki Marine Biological Station and the Sugashima Marine Biological Station and used for experiments.

The eggs were obtained by injection of an isotonic KCl solution and were washed once with sea water. The oxygen uptake was measured both with the Cartesian diver respirometer and the Warburg manometer. The diver technique was essen-

tially the same as that described by Holter (1943). The divers used in the present experiments were of standard type, having an air volume of 8–10 μl. They were charged in the usual manner. The Warburg flask constant was 0.33–0.65. Filtered sea water was used as the physiological medium throughout the experiments. As the measurements were taken at about room temperature, the diver and the flask reached their thermal equilibrium so quickly that it was possible to begin measurements in less than 20 minutes after shedding. The number of eggs was used as reference for the diver measurement, while total nitrogen was employed for the manometric method. To observe the respiratory change at fertilization, the divers were charged as described by Borei (1948b) and Nakano (1953). The unfertilized eggs were placed in the neck of the diver, and an alkali solution at the bottom. When a silicone-coated diver was used, the danger of premature fertilization was considerably reduced. While the diver was observed through the horizontal microscope, an overpressure was applied until eggs and sperm were mixed by the use of Claff's device (1949). This method permitted continuous measurement following fertilization. For the manometric method, the sperm placed in the side arm was added to the egg suspension in the main chamber after an appropriate time. After completing the measurements, the eggs were removed from the diver and the flask

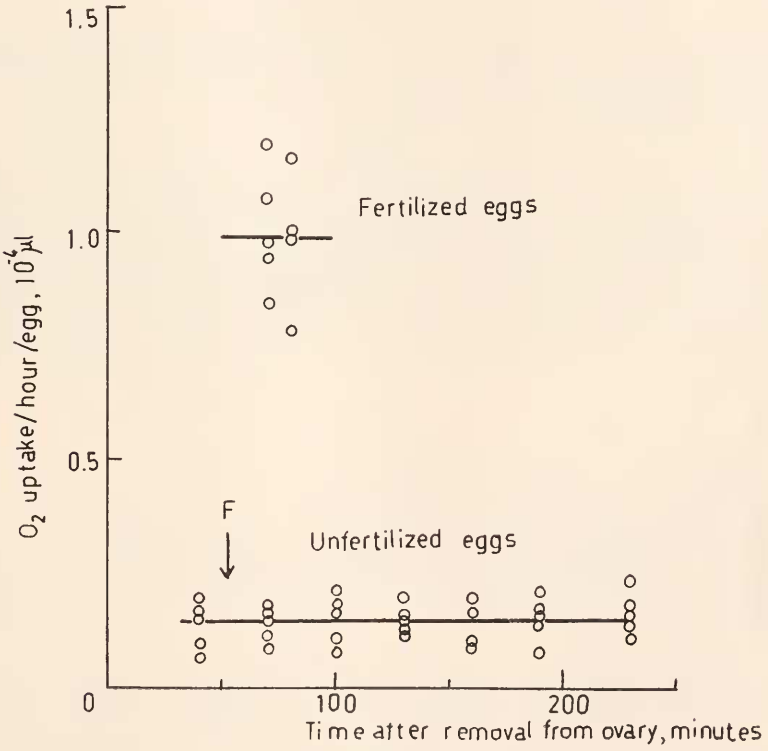

FIGURE 1. Oxygen uptake of eggs of *Pseudocentrotus depressus* before and after fertilization. F, addition of spermatozoa. Temperature, 18° C.

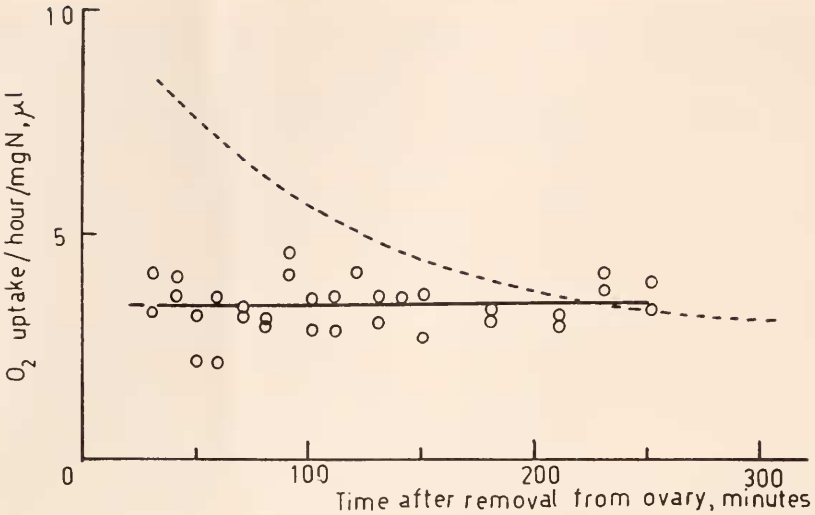

FIGURE 2. Oxygen uptake of unfertilized eggs of *Pseudocentrotus*. Temperature, 20° C. Dotted line is redrawn from Borei (1948a) for comparison.

and the percentage of fertilization was examined. Only fertilization above 90% was taken into consideration for results.

RESULTS

Figure 1 presents five diver technique experiments with *Pseudocentrotus* eggs. There is no gradual decline in oxygen uptake following removal from the ovary. A rather low rate of oxygen uptake is maintained for more than four hours from the time the first measurements were taken. The rate of oxygen uptake in fertilized eggs, however, is much higher than that of unfertilized eggs. Figure 2 shows two

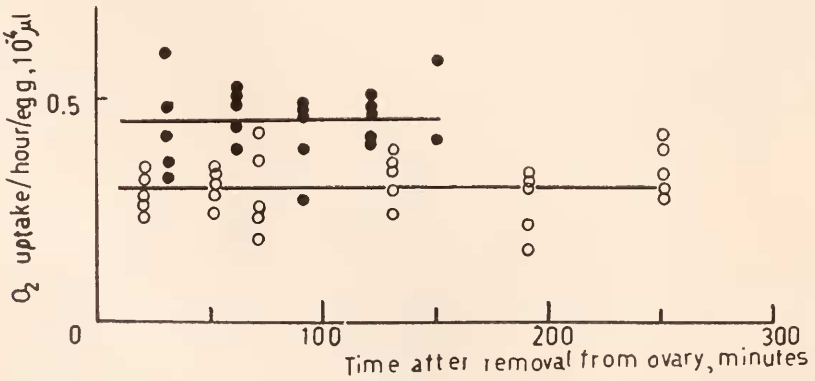

FIGURE 3. Oxygen uptake of unfertilized eggs of *Anthocidaris crassispina* (●) and *Hemicentrotus pulcherrimus* (○). Temperature, 30 and 18° C., respectively.

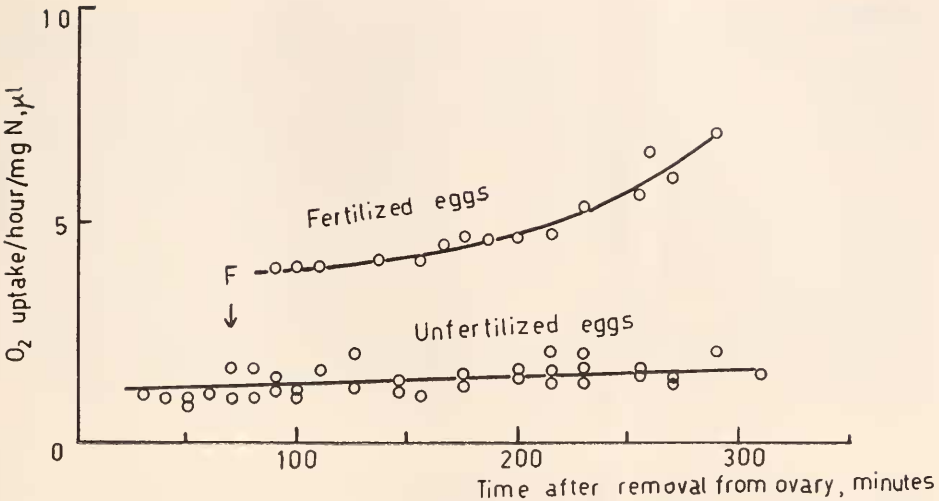

FIGURE 4. Oxygen uptake of *Hemicentrotus* eggs before and after fertilization. F, addition of spermatozoa. Temperature, 10° C.

manometric experiments with *Pseudocentrotus* eggs, neither one showing decline in oxygen uptake of unfertilized eggs.

The same results were obtained with *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*. It is clear from Figure 3 that oxygen uptake of unfertilized eggs immediately after shedding is low and remains so rather constant for three hours or more. When the eggs are fertilized, oxygen uptake increases sharply and the exponential increase starts as shown in Figure 4. These observations are in perfect agreement with those of previous reports of several investigators.

In Borei's experiment with *Psammechinus miliaris* (Z-form), the average rate of oxygen uptake in aged, unfertilized eggs and fertilized eggs is 0.47×10^{-4} and 1.84×10^{-4} µl./egg/hour, respectively. The values in the present experiment check well with those of Borei's experiment, except for the low rate in unfertilized eggs immediately after shedding.

DISCUSSION

The result of the present experiment seems to be in contradiction to Borei's finding (1948a, 1949). The respiratory level of unfertilized eggs turns out to be low even immediately after shedding and there is no gradual decline in oxygen uptake with time after shedding. In eggs of the species used, the sudden increase in oxygen uptake may occur at fertilization under natural conditions. These results seem to suggest that such a phenomenon may yet be important for the understanding of the fertilization mechanism, especially with regard to the activation of enzyme systems. Although there is a good deal of investigation about the mechanism of the sudden increase of oxygen uptake at fertilization in sea urchin eggs, nothing can be said with certainty. Runnström (1930) suggested that structural changes at the time of fertilization may induce contact between enzymes and substrates in respiratory chains of the egg. He also proposed a hypothesis that in

unfertilized eggs an active cytochrome oxidase is present but only partly utilized (Runnström, 1956). In accord with this view, Maggio (1959) demonstrated the increase in the activity of cytochrome oxidase after fertilization. Other critical evidences that the cytochrome oxidase system was operative in unfertilized sea urchin eggs were obtained by Robbie (1946) and Black and Tyler (1959a, 1959b). Recently, Gonse (1960) compared the respiratory level of unfertilized eggs with that of fertilized eggs and suggested that no major change in the enzyme system is involved at the time of fertilization. As to the low respiratory level in unfertilized eggs, there is another possibility that they may contain some inhibitors of cellular oxidation (Runnström, 1949). In fact, Maggio and Monroy (1959) showed an inhibitor of cytochrome oxidase in the supernatant of egg homogenates, whose nature is still obscure. At present it may be too early to draw any conclusion concerning the mechanism of respiratory change occurring at fertilization, but the present result may justify further efforts toward its elucidation.

The authors wish to acknowledge with gratitude the cooperation of Mr. Hideo Tomita, of Nagoya University, in the experiments.

Summary

The respiratory level of sea urchin eggs was measured before and after fertilization, using three Japanese species, *Pseudocentrotus depressus*, *Hemicentrotus pulcherrimus* and *Anthocidaris crassispina*. The rate of oxygen uptake of unfertilized eggs is low even immediately after shedding, and it remains constant for more than three hours. Upon fertilization, the rate of oxygen uptake of the eggs increases sharply, and the increase may occur under natural conditions.

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